

EP 23355 (6)

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

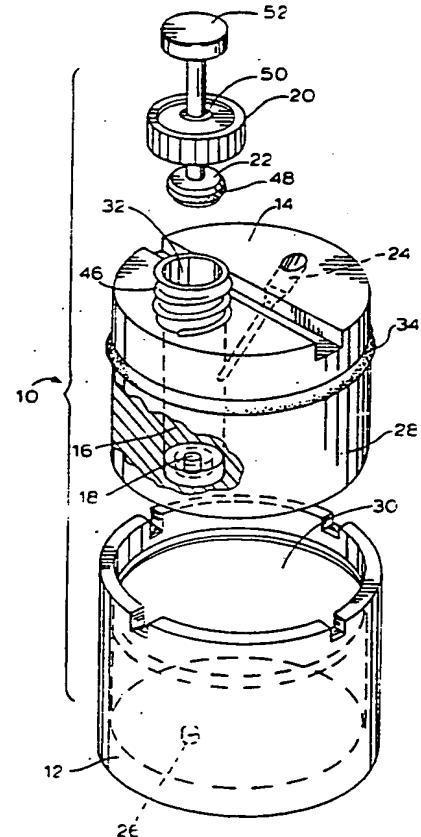
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 35/00, B01L 3/00		A1	(11) International Publication Number: WO 94/04929 (43) International Publication Date: 3 March 1994 (03.03.94)
(21) International Application Number: PCT/US93/07951			(74) Agents: BUONAIUTO, Mark, J. et al.; One Baxter Parkway, Deerfield, IL 60015 (US).
(22) International Filing Date: 24 August 1993 (24.08.93)			
(30) Priority data: 07/934,825 24 August 1992 (24.08.92) US			(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(71) Applicant: BAXTER DIAGNOSTICS INC. [US/US]; One Baxter Parkway, Deerfield, IL 60015 (US).			Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(72) Inventors: COAKLEY, Joseph ; 8500 Cutler Way, Sacramento, CA 95828 (US). GODSEY, James ; 101 Summer Shade Court, Folsom, CA 95630 (US). SHERMAN, David ; 2710 Stonecreek Drive, Apt. #183, Sacramento, CA 95833 (US).			

(54) Title: SEALABLE VESSEL FOR CONTAINING AND PROCESSING ANALYTICAL SAMPLES

(57) Abstract

A sealable container for holding an analytical sample for processing is disclosed. The container includes a body (12) with a dispensing port (26) and an insert member (14) slidably received in the body (12). The insert member and the body are movable relative to each other between a first position, in which the outlet port (18) is substantially aligned with the dispensing port (26), and a second position, in which the outlet port is substantially out of alignment with the dispensing port. Sealing means (48), which may take the form of O-rings between the insert member and the body, provide a substantially airtight, hermetic seal between a reaction chamber formed in the insert member and the body so that the reaction chamber can be sealed or isolated from the outside environment. The container is particularly adapted to contain the processes of nucleic acid amplification and to thereby reduce the problems of sample crosscontamination encountered in such processes. A method of processing an analytical sample using such a container is also disclosed.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

SEALABLE VESSEL FOR CONTAINING
AND PROCESSING ANALYTICAL SAMPLES

FIELD OF THE INVENTION

5 This invention relates generally to containers for holding analytical samples and added reagents in a sealed environment, and is particularly directed to sealed vessels for containing the processes of target nucleic acid amplification in isolation from the
10 outside environment.

BACKGROUND OF THE INVENTION

Modern medical diagnostic tests rely upon the isolation and identification of nucleic acid sequences. Nucleic acids are the components of the genetic
15 material of organisms and are arranged sequentially in long strands to form molecules such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). For example, in order to determine whether a patient is suffering from an illness associated with a particular virus, tests
20 have been developed to identify one or more nucleic acid sequences unique to that virus. These tests rely on the fact that probes complementary to the target nucleic acid sequence will selectively bind or hybridize to the target sequence. The probe is
25 chemically labelled so that it can be detected by one or more analytical methods, such as fluorometry. If a sample contains the target nucleic acid sequence, the probe will bind to the target and the presence of the bound probe can be used to confirm the presence of the
30 target sequence.

Because the target molecule may be present in extremely small quantities in many test samples, processes have been developed to increase or "amplify"

5 replication ("SSR"), polymerase chain reaction ("PCR"),
and the transcriptional amplification system ("TAS"),
and several variations thereof. Basically, such
10 methods produce copies of the nucleic acid in multiple
reiterative steps such that the copies produce further
copies in an exponential fashion. In this way, small
amounts of nucleic acids can be rapidly amplified by a
factor of more than one million, thereby producing
sufficient quantities of target needed for detection in
diagnostic tests. For a general discussion of
15 amplification techniques, the reader is referred to
U.S. Patent No. 4,683,202 (Mullis), which is
incorporated herein by reference and made a part
hereof.

20 Although such target amplification procedures are
extremely powerful, several drawbacks have arisen with
their use. The most significant problem is so-called
sample "cross-contamination," that is, the
contamination of one sample with amplified target from
previous amplification procedures. Reagents used in
25 current amplification methods can also be contaminated
in this manner. Because of the power and specificity
of amplification techniques, even extremely small
amounts of nucleic acid carried in aerosols created
during normal laboratory procedures or present in trace
30 amounts on laboratory equipment can contaminate a
sample and result in false positive results, seriously
jeopardizing the reliability of diagnostic tests. In
extreme situations, contamination problems have become

so severe and widespread that it has been necessary to relocate entire laboratories.

5 Laboratories have developed various methods to help minimize cross-contamination. One approach is to store separate, small aliquots of reagents and to dispense these reagents using so-called "positive displacement" pipettes, so that the reagents and sample do not contact the outside environment before or during the amplification reaction. Other approaches include 10 physically separating amplification reactions from other laboratory processing steps and/or pre-treating reagents with ultraviolet light to destroy nucleic acid fragments present in the reagents as a result of contamination.

15 These techniques for preventing sample and reagent cross-contamination suffer from a number of drawbacks. Physical separation makes it more difficult to perform diagnostic procedures in a single instrument, since amplification must be confined to a separate area -- or 20 even a separate room -- in the laboratory. Likewise, pretreatment with UV light does not guarantee that there will be no cross-contamination, since the reagents may potentially be exposed to contamination after such pretreatment but prior to amplification.

25 Chemical contamination control techniques are also known. Such techniques include so-called "pretreatment," in which DNA sequences are chemically modified during amplification. As a result, DNA copies made by amplification are chemically unique from the 30 target. If these unique copies have contaminated a sample, they can be selectively destroyed prior to each new test without destroying the target. "Post-treatment" techniques are also known, in which the DNA

strands are cross-linked after amplification, making them chemically incapable of being amplified.

Such chemical contamination control techniques also have drawbacks associated with their use. The 5 principal drawback is that such techniques tend to reduce the sensitivity of detection, either by reducing the number of amplified copies or by rendering the amplified copies more difficult to detect with probes. Thus, the existence of these techniques does not 10 eliminate the need for physical isolation of the amplification reaction, particularly when high sensitivity of detection is desired.

Containers for physically isolating analytical samples are also known. For example, U.S. Patent No. 15 2,961,228 to Moore discloses a crucible to prevent a sample from exposure to the atmosphere prior to testing. However, this device is not adapted for use in modern diagnostic procedures and, in particular, procedures which involve nucleic acid amplification.

Accordingly, a need exists for an analytical sample 20 container which can maintain the processes of target nucleic acid amplification (including sample lysing, addition of amplification reagents, and amplification) in substantial isolation from the outside environment, thereby helping to reduce sample and reagent cross-contamination. Further, a need exists for a sample 25 container which permits the required amplification reagents to be added to the sample while still maintaining the sample in isolation from the outside environment. A need also exists for a container which 30 may be used in a single, automated or semi-automated instrument, but which is relatively simple in design and easy to manufacture.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a sample container for clinical specimens which maintains the specimen and added reagents in isolation from the outside environment to prevent specimen and reagent cross-contamination.

It is a further object of this invention to provide a sample container which is designed to permit the addition of reagents (and, preferably, the reagents required for nucleic acid amplification) while still maintaining the sample and reagents in isolation from the outside environment.

It is a further object of this invention to provide a sample container which is relatively simple in design and easy and inexpensive to manufacture so that the container may be disposed of after use, thereby further reducing contamination problems.

It is a further object of this invention to provide a sample container which is designed so that precise quantities of the sample and, when amplification is performed, amplified target, may be reliably dispensed from the container for further processing in diagnostic procedures.

It is a further object of this invention to provide a sample container which is adapted for use in an automated or semi-automated instrument.

It is a further object of this invention to provide a sample container which simplifies sample handling and processing.

It is a further object of this invention to provide a sample container made of materials which are capable of withstanding the varying temperature conditions used for many amplification reactions.

5 received in the body. The insert member defines a reaction chamber with an outlet port. The insert member and the body are movable relative to each other between a first position, in which the outlet port is substantially aligned with the dispensing port, and a second position, in which the outlet port is substantially out of alignment with the dispensing port. An analytical sample may be introduced into the reaction chamber through a closeable inlet means which communicates with the reaction chamber. In order to provide a substantially airtight, hermetic seal between the reaction chamber and the outside environment when the inlet into the reaction chamber is closed and the outlet means and the dispensing port are moved out of alignment, sealing means are provided between the insert member and the body. The insert member may include a generally cylindrical mating member adapted to be slidably and rotatably received in a corresponding generally cylindrical recess in the body. The container of the present invention may also be used to practice a method of processing an analytical sample, as will be described more fully hereinafter.

30 The foregoing features and advantages of the present invention will be more readily understood upon consideration of the following detailed description, taken in conjunction with the accompanying drawings, in which:

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is an exploded perspective view of a sample container made in accordance with the present invention;

5 FIG. 2 is a perspective view of an alternative embodiment of the sample container made in accordance with the present invention;

10 FIG. 3 is a view similar to that of FIG. 2 showing
a plurality of sample containers held in a tray
configuration;

FIG. 4 is a sectional view of the sample container showing the sample being held in the reaction chamber:

15 FIG. 5 is a view similar to that of FIG. 4, showing the addition of reagents through an auxiliary access port into the reaction chamber:

FIG. 6 is a view similar to that of FIG. 4, showing how the auxiliary access port may be hermetically sealed after the addition of reagents:

20 FIG. 7 is a top view of the sample container taken along the line 7--7 in Fig. 4, showing how the insert member may be rotated within the container body:

FIG. 8 is a view similar to that of FIG. 4, showing how the sample and reaction mixture may be dispensed from the container through a dispensing port: and

25 FIG. 9 is an enlarged cross-sectional view of a portion of the container, showing the reaction chamber outlet port axially aligned with the body dispensing port.

DETAILED DESCRIPTION

30 Figs. 1 and 2 show the general arrangement of a sample container 10 made in accordance with the present

invention. This Specification describes a preferred form of the invention, in which the sample container is used to hold an analytical sample and contain the process of nucleic acid amplification. However, it 5 will be understood that the container may be used to perform any of various processing steps commonly performed on analytical samples and well known to those skilled in the art. Such processing steps include, by way of example, chemical and immunochemical reactions, 10 labelling and detection, and the like.

As best seen in Fig. 1, in general the container 10 includes a body 12 and an insert member 14 adapted to be received within the body 12. The insert member 14 15 includes a reaction chamber 16 in which the sample and any added reagents are held during sample handling and processing. A threaded cap and slidable plunger 22 may be used to close the inlet into the reaction chamber 16. Likewise, an auxiliary port 24, which communicates with the reaction chamber 16, may be provided for the 20 addition of the sample and/or other reagents during processing. The container 10 is constructed so that the reaction chamber may be completely sealed from the outside environment during sample processing. Such an arrangement is particularly useful in helping to 25 prevent sample and reagent cross-contamination when performing nucleic acid amplification reactions.

By aligning an outlet port 18 from the reaction chamber 16 with a dispensing port 26 on the body 12, fluids may be dispensed from the reaction chamber 16 30 for further processing and/or disposal. For example, after amplification reactions have taken place, aliquots of sample containing the amplified target may be transferred to a microtitre well for detection using conventional diagnostic methods. This dispensing step

is preferably performed in a location remote from that where the amplification reaction takes place so that contamination of subsequent samples from aerosols that may be created during dispensing is avoided.

5 The container of the present invention may be used as part of an automated or semi-automated instrument designed to handle and process analytical samples. Such an instrument could be adapted to perform some or 10 all of the steps described hereinafter, including manipulating the various components of the sample container.

15 If the container is used in an automated or semi-automated instrument, the container could be manually or mechanically moved to a remote position before the outlet port and dispensing port are rotated into alignment for dispensing.

20 Referring again to Fig. 1, the construction and operation of the container 10 will now be described in further detail. The basic components of the container are the body 12 and the insert member 14. In a preferred form of the invention, these components are 25 molded from suitable plastics using conventional injection molding techniques. However, it will also be understood that other methods of manufacture may also be used. For example, the components could be molded by other means or they could be machined or formed from other well known plastics or materials.

30 To date, success has been had with a clear polycarbonate known as Lexan®, made by General Electric Co. In this regard, it is preferred that the material be optically transparent so that the laboratory technician may view the processes taking place in the reaction chamber. In addition, since a number of amplification reactions (in particular, 3SR) require

amplification reactions or other appropriate
approved medical grade plastics.

10 The insert member 14 is designed to be slidably received in the body 12. In a preferred configuration, the insert member 14 has an integrally formed, generally cylindrical mating member 28 which is sized to fit in a corresponding generally cylindrical recess 30 in the body. Once inserted, the insert member 14 is free to rotate relative to the body about an imaginary vertical line passing through the center of cylindrical mating member 28. It will be understood, however, that other configurations which permit the insert member and body to slide relative to each other may be used. In this regard, it is not essential that the mating member and recess be generally cylindrical as long as they are adapted for relative movement.

25 A reaction chamber 16 is formed in the insert member 14 by molding, forming, drilling, or the like. The reaction chamber may also be an insert which is made separately and then inserted into an appropriate aperture in the insert member. In that event, the reaction chamber insert may be made of the same or a different material as the other components of the container. A particularly preferred material for an insert of this type is polypropylene. In one form of the invention, the reaction chamber is smooth-walled and generally cylindrical in shape with a conical taper

adjacent the outlet port. As best seen in Figs. 2 and 9, in this arrangement, the reaction chamber 16 tapers to a smaller diameter, which is sized to closely match the upper diameter of the dispensing port 26 formed or drilled in the body, thereby facilitating smooth fluid flow through the aligned ports. In an alternative embodiment, the reaction chamber has two tapers, with the diameter of the chamber decreasing to form a narrower cylindrical region below the higher taper. In this arrangement, the slidable plunger may be designed so that it fits snuggly in the narrower cylindrical region beneath the first taper. However, as described in greater detail below, the chamber may be formed in any number of shapes, including rectangular, conical, or irregular. Indeed, as is also described in greater detail below, the container may be designed so that the reaction chamber actually changes in size and/or shape during the course of processing the sample.

The reaction chamber is preferably designed to contain an analytical sample during the processes of nucleic acid amplification; however, it will be understood that it may be shaped to hold the sample during any desired handling or processing step used in diagnostic methods. In a preferred form, the reaction chamber is designed to adequately contain the amount of fluid typically handled during amplification reactions -- typically between about 20 μ l and about 150 μ l and, more particularly, between about 40 μ l and about 103 μ l of fluid. However, it will be understood that the chamber may be designed to hold any desired quantity of fluid.

The reaction chamber 16 is also preferably equipped with an inlet 32 and an outlet port 18. By rotating the insert member 14 relative to the body, the outlet

moving both simultaneously. Accordingly, references
herein to "movement" or "rotation" of the insert
member, mating member, or the body should be understood
10 to include any means by which such relative movement is
accomplished. When the outlet port 18 is moved to a
first position, in which it is substantially aligned or
communicating with the dispensing port 26, fluid may be
dispensed from the reaction chamber 16 for further
15 processing.

An inlet 32 may be used to introduce sample
and/or reagents into the reaction chamber 16. In one
preferred form of the invention, a closure means in the
form of a threaded, sealed cap 20 can be used to close
20 the inlet 32 in a substantially airtight manner.
However, it will be understood that other conventional
closure means may also be used, such as, by way of
example, a septum, a self-sealing septum, a snap-
fitable lid, or simply a plunger with a radial seal.
25 It is also preferred that the reaction chamber be
equipped with an auxiliary port 24 which communicates
with the reaction chamber 16 at some point along its
length. Such a port may be drilled or otherwise formed
(e.g., by molding) in the insert member. When an
auxiliary port is provided, it is preferred that the
30 opening from the auxiliary port to the reaction chamber
be as close to the bottom of the reaction chamber as
possible, while still leaving sufficient volume to
contain the sample/reagent solution. It is also

preferred that the interior walls of this port be as smooth as possible to facilitate flow of reagents.

In the embodiment depicted in Fig. 1, this auxiliary port 24 is formed so that it passes at an angle from the top of the insert member 14 into the reaction chamber. This angle should be sufficiently steep to permit fluid in the auxiliary port to flow readily under the influence of gravity into the reaction chamber; preferred angles are between about 35 and 80 degrees from horizontal and, more particularly, about 60 degrees from horizontal. This auxiliary port may be used to introduce sample and/or reagents into the reaction chamber 16. In a preferred form of the invention, this auxiliary port 24 is used to introduce reagents used to perform nucleic acid amplification reactions into the reaction chamber.

An important aspect of the present invention is the ability to seal the reaction chamber 16 in a substantially hermetic, airtight manner from the outside environment during and after the various sample-processing steps. This is particularly important to prevent contamination when performing nucleic acid amplification reactions; however, it will be understood that it is often desirable in other diagnostic procedures to isolate the sample and/or reagents from the outside environment during various processing steps.

This isolation or sealing may be accomplished in a number of ways. In one form of the invention, the container is provided with a plurality of sealing means. The first sealing means may take the form of a radial seal, such as an O-ring 34. The O-ring may be made of any appropriate elastomer, such as EPDM, for example, or of rubber. The O-ring 34 is affixed around

5 the outer wall of the mating member 28. Insertion of
the O-ring may be accomplished manually by stretching
the elastomeric O-ring around the mating member 28 in
an interference fit relationship or by insert molding
the O-ring into the groove as part of an injection
10 molding process. It will also be understood that other
radial-type sealing means may be used, including u-cup
shaped seals, gaskets, and the like.

15 Referring simultaneously to Figs. 1 and 4,
the O-ring is sized so that when the mating member 28
is inserted into the interior of the cylindrical recess
30 formed in the body 12, the O-ring will engage the
interior wall of the cylindrical recess 30 in an
interference fit relationship, thereby effecting a
substantially airtight, hermetic seal between the side
20 walls of the mating member 28 and the interior wall of
the body 12.

25 Referring again to Fig. 1, a dispensing port
26 is formed or drilled in the body for dispensing
fluid from the reaction chamber 16 when the outlet port
18 is rotated into alignment with the dispensing port
26. Since this dispensing port 26 communicates with
the outside environment, a second sealing means is
provided to isolate the outlet port 18 of the reaction
30 chamber 16 from the dispensing port 26 when the ports
are out of alignment.

The configuration of this second sealing
means is best seen by reference to Fig. 9. This
sealing means takes the form of a second O-ring 40.
Success has been had with an O-ring bearing the

designation AS-568-020, manufactured by Apple Rubber Products. This second O-ring 40 is affixed to a corresponding O-shaped recess 42 formed in the bottom wall 44 of the mating member 28 in the manner described above. When the mating member 28 is inserted into the cylindrical recess 30 formed in the body 12, the second O-ring 40 is pressed into engagement with the bottom wall of the cylindrical recess 30 to effect an airtight, hermetic seal when the outlet port 18 of the reaction chamber 16 is moved out of alignment with the dispensing port 26. (The outlet port 18 and dispensing port 26 are shown in alignment in Fig. 9; Fig. 4 illustrates the outlet port 18 and dispensing port 26 in an out-of-alignment position.) It will be understood that it may be necessary to press or force the second O-ring 40 into a sealing engagement with the bottom wall of the cylindrical recess 30 to effect the necessary seal. This may be accomplished either by applying a continuous downward force to the mating member 28 or by locking the mating member 28 to the body 20 so that the second O-ring is held in sealing engagement with the bottom wall of the recess 30.

Since the mating member 28 is received within the body 12, it will be seen that fluid held within the reaction chamber 16 will come into contact with the inner bottom wall 62 of the body 12. This is not undesirable, since such fluid may help to lubricate the second O-ring 40 and thereby assist in maintaining a substantially airtight seal. However, it will also be seen that when the mating member 28 is rotated relative to the body 12, fluid will be deposited along the inner bottom wall 62. Since this fluid could conceivably pass beyond the second O-ring, it is important that the first O-ring (identified by reference numeral 34 in Fig. 1) be present to provide a further barrier against

release of the contents, which can result in contamination of the laboratory environment. It is also important that relative movement of the mating member 28 and the body be kept to a minimum before and during the amplification reaction so that fluid deposited on the inner bottom wall 62 does not pass beyond the second O-ring where it could be exposed to the outside environment through the dispensing port formed in the bottom of the body. Indeed, it is preferred that the mating member and body be kept in the same relative position during the addition of amplification reagents and during the entirety of the amplification reaction; movement should only occur in order to align the outlet port and the dispensing port for the dispensing step.

It should also be understood that the position and configuration of the sealing means in the drawings and accompanying description is exemplary only. In this regard, it will be apparent that additional sealing means could be used, and that the sealing means could be affixed to the body, rather than the insert member.

Referring again to Fig. 1, means are also provided to seal or close the inlet 32 into the reaction chamber 16 from the outside environment. This closure means may take the form of a threaded cap 20 which engages corresponding threads 46 on the top of the insert member 14. In a still further preferred form of the invention, the cap 20 is equipped with an associated plunger 22 adapted to be slidably received within the reaction chamber 16. Success has been had with a plunger made of Delrin®, an acetal. The plunger 22 passes through a seal 50 in the cap 20 to prevent leakage. The plunger is also equipped with one or more

circumferential elastomeric sealing lobes 48 sized and shaped to be inserted into the reaction chamber in an interference fit relationship, thereby further sealing the reaction chamber 16 from the outside environment.

5 It will also be understood that other closure or sealing means may be used to close the inlet, such as, by way of example, a septum, a self-sealing septum, or a plunger with a radial seal but without an associated cap. In this regard, the use of a capless plunger 10 simplifies the container and makes it possible to manufacture the container more easily and inexpensively.

15 The container may be equipped with one or more auxiliary ports 24, as shown in Fig. 1. In that event, the plunger 22 may serve an additional purpose. By pressing on the plunger handle 52, the plunger 22 can be moved downwardly into the reaction chamber 16. (It will be understood that in referring to this embodiment the term "reaction chamber" is used to mean 20 the volume below the plunger seal. Accordingly, the reaction chamber actually decreases in volume as the plunger 22 is moved downwardly.) When the plunger 22 is moved below the level where the auxiliary port 24 makes entry into the reaction chamber 16, the plunger 25 22 effects a seal between the reaction chamber 16 and the auxiliary port 24 such that there is no longer communication between the two. When this is done, and the outlet port 18 is in a position out of alignment with the dispensing port 26 on the body, a 30 substantially airtight seal is created between the reaction chamber 16 and the outside environment. As noted above, this is the preferred containment environment for performing many diagnostic procedures, especially nucleic acid amplification reactions.

5 eliminate much of the material
the embodiment illustrated in Figs. 1 and 4-9. This is
desirable for a number of reasons. First, a reduction
in the amount of material reduces the overall cost of
manufacturing the container. Second, a reduction in
material makes it easier to rapidly and efficiently
10 heat and cool the reaction chamber and its contents.
This is particularly important when performing
amplification reactions (and, in particular, 3SR),
since it is often necessary to take the sample and/or
reagents through cyclical temperature changes.
15 Preferably, a reduced-material design is created by
injection molding, as described above.

20 Referring now to Fig. 2, it will be seen that
in this embodiment the insert member 14 is no longer a
solid cylindrical block. As a result, the generally
cylindrical mating member 28 is greatly reduced in
size. The reaction chamber 16 is formed in the insert
member 14 and is supported by a plurality of struts 53
molded around its periphery. Similarly, the auxiliary
port 24 is formed in an integral upright wall 54 of the
25 insert member 14.

30 The reaction chamber 16 is sealed from the
outside environment in a manner similar to that
described above. A first sealing means in the form of
an O-ring or other radial seal (not shown in Fig. 3)
may be affixed in a manner similar to that described
above to the outer cylindrical wall 56 of the mating
member 28 to engage the inner wall of the body in an
interference fit relationship. Likewise, a second
sealing means in the form of a second O-ring (not

shown) may be affixed to the bottom wall of the mating member surrounding the periphery of the outlet port 18 to effect a face seal between the outlet port 18 and the bottom of the cylindrical recess in the body, as described above. A cap 20 and associated plunger 22 may also be provided as described above to seal the inlet into the reaction chamber 16 and, on moving the plunger 22 downward, to close the auxiliary port.

Fig. 3 illustrates a "cartridge" for holding a plurality of containers. In this embodiment, a plurality of insert members 14 are inserted into corresponding cylindrical recesses 30 in tray 58. The bottom of tray 58 is equipped with a dispensing port (not shown) for each insert member 14. In this way, a plurality of samples may be processed in separate containers at the same time -- an important advantage in most diagnostic procedures, particularly those performed in clinical and hospital laboratories.

Figs. 4-8 illustrate the sequence of steps which may be followed in using the container of the present invention to hold and process an analytical sample. In the description which follows, it will be assumed that a nucleic acid amplification reaction will be performed on the sample; however, as noted above, it will be understood that the container may also be used to perform other diagnostic procedures in which isolation from the outside environment is desired.

Prior to the introduction of sample and/or initial reagents into the container, the mating member 28 is rotated to a position in which the outlet port 18 of the reaction chamber 16 is substantially out of alignment with the dispensing port in the body. Fig. 4 illustrates the container after the sample and initial reagents 60 have been placed in the reaction chamber

16. Generally, the initial reagents include lysing agents which break down the cell membranes or protein coatings of the sample, thereby releasing nucleic acids from the sample so that they may be amplified. The 5 sample and lysing reagents may be introduced into the reaction chamber by removing the threaded cap 20 and associated plunger 22 and pipetting or otherwise transporting the sample and reagents through the inlet 32, or by piercing a sealed septum covering the inlet 10 with a needle or probe. Alternatively, the lysing reagents may be stored in the reaction chamber in a dried or lyophilized form prior to introduction of the sample and then reconstituted upon addition of sample. In total, approximately 40 μ l of sample and lysing 15 reagents are present when performing 3SR amplification.

In a step which is not shown in the drawings, immediately after the sample and/or initial reagents are added, the plunger is moved downwardly below the level of the auxiliary port, thereby closing it from 20 communication with the reaction chamber. This helps to immediately isolate the sample from the outside environment, thereby reducing contamination problems. It will also be understood that the auxiliary port may 25 also be closed by other means, including a separate valve, for example. Of course, if no auxiliary port is provided, this step is unnecessary.

To prepare the sample for the addition of amplification reagents, the sample may be subjected to temperature cycling. For example, when 3SR 30 amplification is used, the container (or cartridge containing a plurality of containers) may be placed in an incubator, where the sample is first heated to a temperature of 50 °C for about 60 minutes, then to 100 °C for approximately 5 minutes, and finally to 42 °C

prior to addition of 3SR amplification reagents. It will be understood that other temperature conditions well known to those skilled in the art may be necessary for other amplification reactions.

5 Fig. 5 illustrates the addition of amplification reagents through the auxiliary port 24. In a step which occurs prior to that shown in Fig. 5, the reagents to be added are conveyed into the auxiliary port while the plunger is still in its lowered position covering or closing the opening 23 which leads from the auxiliary port into the reaction chamber. The plunger 22 thereby prevents communication between the auxiliary port and the reaction chamber. It will be understood, however, that these reagents may 10 also be added through the inlet 32 if no auxiliary port is provided. For example, the reagents could be added by removing the cap 32 or by piercing a self-sealing septum covering the inlet with a needle or probe.

When one or more auxiliary ports are 20 provided, the amplification reagents are transported by pipette or other means, either manually or robotically, into the auxiliary port 24. For 3SR amplification, the amplification reagents or "cocktail" may include, by way of example, buffers, rNTP's and dNTP's, primers, 25 and other reagents. As shown in Fig. 5, the plunger 22 is then raised above the opening 23 in the auxiliary port 24 so that reagents are allowed to pass under the influence of gravity in the direction of the arrows and into the reaction chamber 16. To maintain a condition 30 in which the reaction chamber is sealed from the outside environment during this step, it is preferred that the reagents be pipetted through the auxiliary port 24 in appropriate quantities so that the reaction chamber 16 is actually sealed from the outside

environment by the fluid passing through the auxiliary port 24 until the auxiliary port may be closed or sealed mechanically, as described below. It is also important that the plunger 22 be raised only long enough for the appropriate metered quantity of reagents to pass into the reaction chamber. Finally, it is important that the level of the sample/reagent mixture not be permitted to rise to the level of the auxiliary port opening into the reaction chamber, where it potentially could be exposed to the outside environment during later processing steps.

Fig. 6 illustrates the next step in the preferred sequence. After the appropriate metered quantity of reagents is allowed to pass into the reaction chamber, and while the fluid seal is being maintained in the auxiliary port 24 by the amplification reagents, the plunger handle 52 is once again depressed to move the plunger 22 downward in the direction of the arrow in Fig. 6. It will be understood that movement of the plunger may be accomplished manually by the laboratory technician or mechanically by means of an automated or semi-automated instrument equipped to mechanically raise and lower the plunger 22 during the various raising and lowering motions described herein. The plunger 22 is moved downwardly past the opening from the auxiliary port into the reaction chamber 16. The lobe 48 on the plunger 22 thereby once again seals or closes the auxiliary port 24 from communicating with the reaction chamber, which, after the plunger is lowered, is the decreased volume located beneath the lobe 48. At this point, with the mating member 28 positioned such that the outlet port 18 is out of alignment or communication with the dispensing port on the body, the reaction chamber 16 is substantially isolated from the outside

environment by an airtight, hermetic seal created by the first O-ring 34, the second O-ring 40, the lobe 48 on the plunger, and the threaded cap 20. The amplification reaction may thus proceed in a completely sealed chamber, thereby significantly reducing the possibility that nucleic acids from other sources will contaminate the sample and interfere with diagnostic test results, or that amplified nucleic acids will be released from the container into the surrounding laboratory environment.

Fig. 7 illustrates the next step in the preferred sequence. After the amplification reaction is complete, the sample now includes amplified quantities of the target sequence (assuming, of course, that the target was present in the sample to begin with). To help reduce the possibility that amplified target will be released in the location where other samples will be subjected to amplification, it is now desirable to transfer the container to another location in order to perform one or more assays for the amplified target. Typically, the container will be transferred to another location where the sample can be dispensed into a microtitre well or other similar analytical vessel. Here it will be subjected to various labelling and detection reactions so that the presence or absence of the target may be detected. Problems associated with exposing the sample to the outside environment are greatly reduced if detection is performed in a remote location designed to inhibit movement of any aerosols back toward the amplification zone (e.g., by means of a positive airflow system).

In order to permit the sample to be dispensed from the container, the mating member 28 is rotated relative to the body 12 about an imaginary axis passing

through the center of the cylindrical mating member 28. (This imaginary axis is indicated by an "x" in Fig. 7 and by the dotted lines in Figs. 4-6.) The mating member 28 is moved or rotated in this manner until the outlet port of the reaction chamber is brought into alignment with the dispensing port on the body. As noted above, this may be accomplished by any means which moves the mating member 28 relative to the body 12. As with actuation of the plunger, the components may be moved either manually by an operator or mechanically in an automated or semi-automated instrument designed to grip one or more of the components. At this point, sample held within the reaction chamber may be dispensed through the outlet port and dispensing port.

Fig. 8 illustrates the use of the plunger 22 to dispense the sample out of the container. Once the outlet port 18 is aligned with the dispensing port 26, downward movement of the plunger 22 in the direction of the arrow in Fig. 8 moves the sample by controlled, positive displacement out of the ports. By sizing and shaping the ports appropriately, downward incremental movement of the plunger 22 can be correlated to precise metered quantities of the sample solution being expelled or discharged from the ports. For many diagnostic procedures, it is preferred that the solution be dispensed in single or multiple increments of 10-15 μ l and, more preferably, about 10 μ l. For this purpose, the plunger may be depressed the correct incremental distances either manually or automatically. It will also be understood that the sample solution may be permitted to exit under the influence of gravity alone or may be expelled by other well-known dispensing mechanisms.

After dispensing is complete, the mating member may be rotated again to position the outlet port and the dispensing port so that they are out of alignment. In this way, the contents of the reaction chamber are sealed, preventing leakage and potential biohazard. The sealed container may then be disposed of in an appropriate fashion.

While the invention has been described in connection with certain presently preferred components and arrangements, those skilled in the art will recognize many modifications to structure, arrangement, portions, elements, materials, steps, and components which can be used in the practice of the invention without departing from the principles thereof.

CLAIMS

WHAT IS CLAIMED IS:

1. A container for holding an analytical sample during processing comprising:

5 a body having a dispensing port,
 an insert member slidably received in the
 body, said insert member defining a reaction chamber
 with an outlet port, said insert member and said body
 being movable relative to each other between a first
10 position, in which the outlet port is substantially
 aligned with the dispensing port, and a second
 position, in which the outlet port is substantially out
 of alignment with the dispensing port,

15 inlet means communicating with the reaction
 chamber for introducing an analytical sample into the
 reaction chamber, said inlet means being closeable, and

20 sealing means between the insert member and
 the body for providing a substantially airtight seal
 between the reaction chamber and the outside
 environment when the insert member and body are in the
 second position and the inlet means is closed.

25 2. The container of Claim 1 wherein the body
 has a generally cylindrical recess formed therein and
 the insert member includes a generally cylindrical
 mating member slidably and rotatably received in the
 cylindrical recess in the body.

30 3. The container of Claim 1 further
 comprising an auxiliary port communicating with the
 reaction chamber and valve means cooperating with the
 auxiliary port for closing the auxiliary port.

4. The container of Claim 1 further
 comprising dispensing means coupled to said reaction

chamber for dispensing fluids within the reaction chamber through the outlet port and the dispensing port when the insert member and the base are in the first position.

5 5. The container of Claim 1 wherein the sealing means comprises a first sealing member between the insert member and the body for maintaining a substantially airtight seal therebetween, and a second sealing member between the outlet port and the body for maintaining a substantially airtight seal therebetween when the insert member and the base are in the second position.

10 6. A container for holding an analytical sample during processing comprising:

15 a body having a dispensing port and a generally cylindrical recess formed therein,

20 an insert member, said insert member including a generally cylindrical mating member slidably and rotatably received in the cylindrical recess in the body, said insert member further defining a reaction chamber, an inlet port, an auxiliary port, and an outlet port, each of said inlet, auxiliary, and outlet ports communicating with the reaction chamber,

25 said mating member and said body being rotatable relative to each other between a first position, in which the outlet port is substantially aligned with the dispensing port, and a second position, in which the outlet port is substantially out of alignment with the dispensing port,

30 first sealing means between the mating member and the recess in the body for maintaining a substantially airtight seal therebetween,

second sealing means between the outlet port and the body for maintaining a substantially airtight seal therebetween,

5 valve means cooperating with the auxiliary port for closing the auxiliary port,

closure means cooperating with the inlet port for closing the inlet port,

10 said first and second sealing means and said valve and closure means providing a substantially airtight seal between the reaction vessel and the outside environment when the mating member and body are in the second position and the valve and closure means are in their closed conditions, and

15 dispensing means coupled to said reaction chamber for dispensing fluids from the reaction chamber through the outlet port and the dispensing port when the mating member and body are in the first position.

7. A method of processing an analytical sample comprising:

20 providing a container for holding an analytical sample, said container comprising a body having a dispensing port, an insert member slidably received in the body, said insert member defining a reaction chamber with an outlet port, said insert member and said body being movable relative to each other between a first position, in which the outlet port is substantially aligned with the dispensing port, and a second position, in which the outlet port is substantially out of alignment with the dispensing port, 25 inlet means communicating with the reaction chamber for introducing an analytical sample into the reaction chamber, said inlet means being closeable, and sealing means between the insert member and the body

for providing a substantially airtight seal between the reaction chamber and the outside environment when the insert member and the body are in the second position and the inlet means is closed;

5 positioning the insert member and the base in the second position;

introducing the analytical sample into the reaction chamber through the inlet means;

10 introducing sample-processing reagents into the reaction chamber through the inlet means;

closing the inlet means;

positioning the insert means and the body in the first position; and

15 dispensing the sample and reagents from the reaction chamber.

8. The method of Claim 7 wherein the body has a generally cylindrical recess formed therein and the insert member includes a generally cylindrical mating member slidably and rotatably received in the 20 cylindrical recess in the body.

9. The method of Claim 7 wherein the container further comprises an auxiliary port communicating with the reaction chamber and valve means cooperating with the auxiliary port for closing the auxiliary port, and wherein reagents are introduced 25 into the reaction chamber through the auxiliary port and the valve means is then closed.

10. The method of Claim 7 wherein the container further comprises dispensing means coupled to 30 said reaction chamber for dispensing fluids in the reaction chamber through the outlet port and the

dispensing port when the insert member and the base are in the first position.

11. The method of Claim 7 wherein the sealing means comprises a first sealing member between the insert member and the body for maintaining a substantially airtight seal therebetween, and a second sealing member between the outlet port and the body for maintaining a substantially airtight seal therebetween when the insert member and the base are in the second position.

10 12. A method of processing an analytical sample comprising:

15 providing a container for holding an analytical sample, said container comprising a body having a dispensing port and a generally cylindrical recess formed therein, an insert member, said insert member including a generally cylindrical mating member slidably and rotatably received in the cylindrical recess in the body, said insert member further defining a reaction chamber, an inlet port, an auxiliary port, and an outlet port, each of said inlet, auxiliary, and outlet ports communicating with the reaction chamber, said mating member and said body being rotatable relative to each other between a first position, in which the outlet port is substantially aligned with the dispensing port, and a second position, in which the outlet port is substantially out of alignment with the dispensing port, first sealing means between the mating member and the body for maintaining a substantially airtight seal therebetween, second sealing means between the outlet port and the body for maintaining a substantially airtight seal therebetween, valve means cooperating with the auxiliary port for closing the auxiliary port, closure means cooperating with the

20
25
30

inlet port for closing the inlet port, said first and second sealing means and said valve and closure means providing a substantially airtight seal between the reaction vessel and the outside environment when the mating member and body are in the second position and the valve and closure means are in their closed conditions, and dispensing means coupled to said reaction chamber for dispensing fluids from the reaction chamber through the outlet port and the dispensing port when the mating member and body are in the first position;

positioning the mating member and the base in the second position;

introducing the analytical sample into the reaction chamber through the inlet;

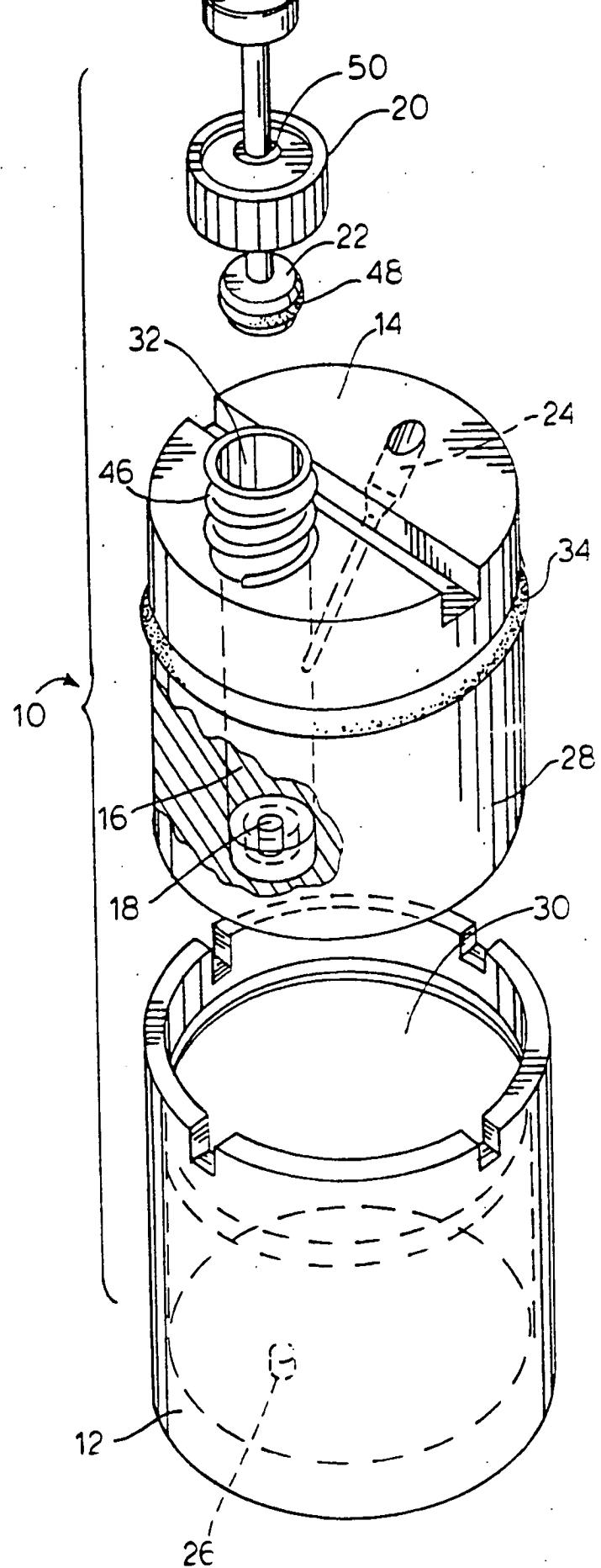
closing the closure means;

introducing sample-processing reagents into the reaction chamber through the auxiliary port;

closing the valve means;

positioning the mating member and body in the first position; and

actuating the dispensing means to dispense the sample and the reagents from the reaction chamber.



214

FIG. 2

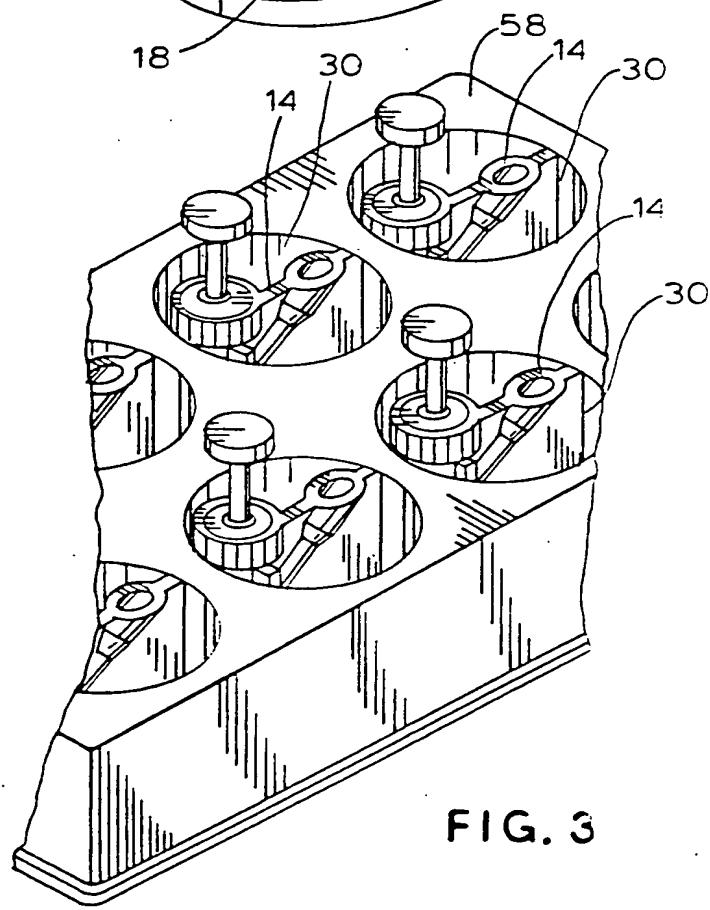
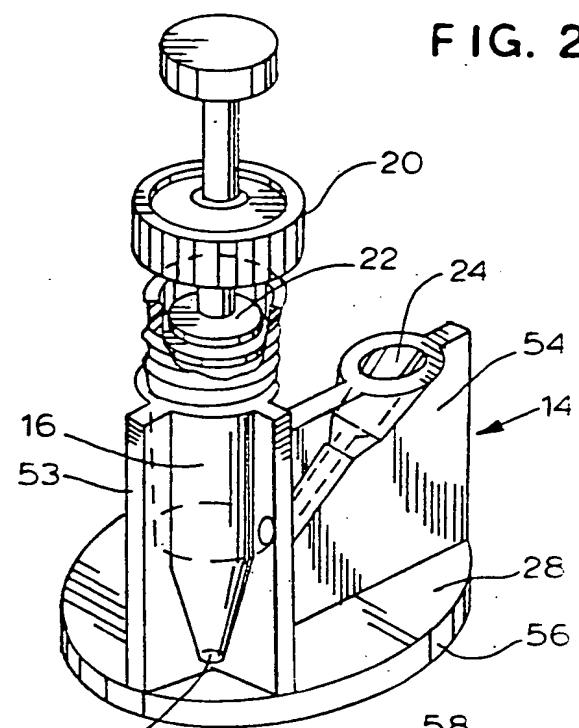
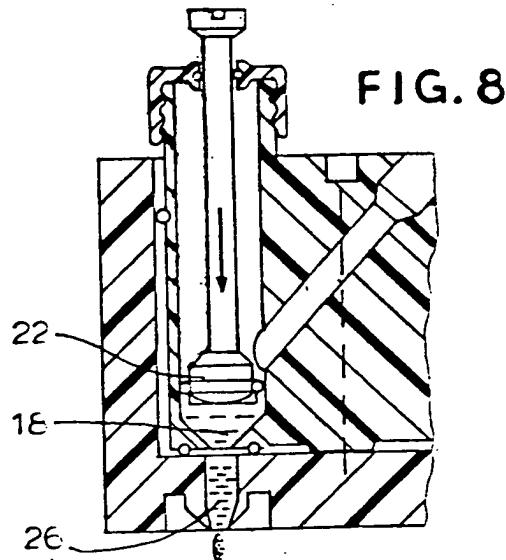
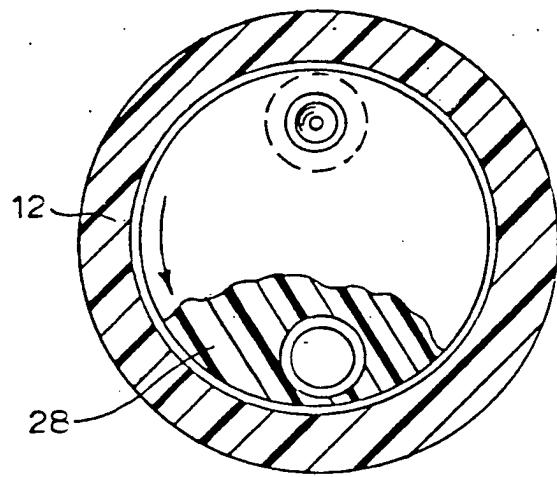
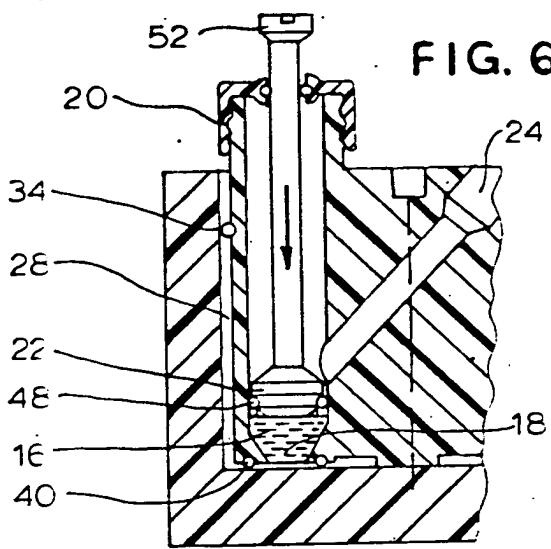
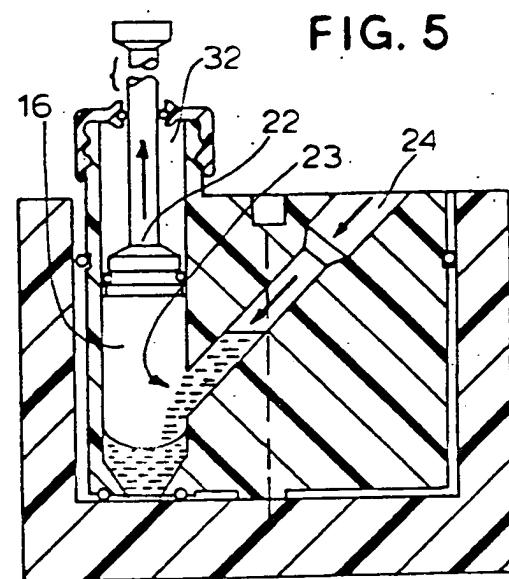
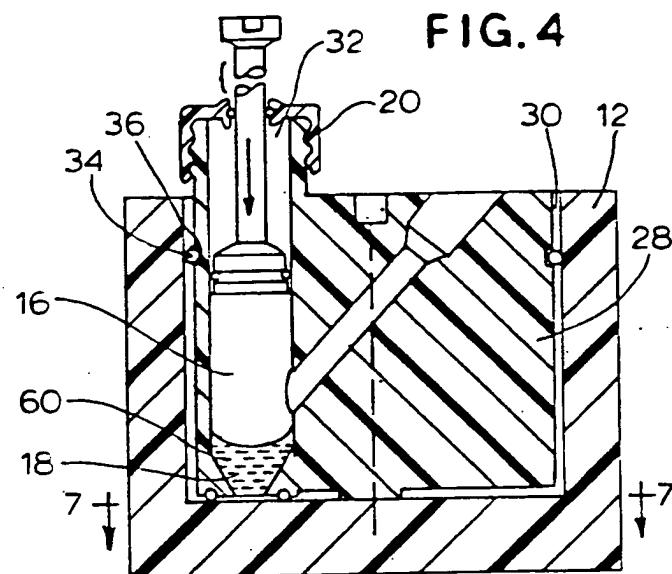


FIG. 3

3/4



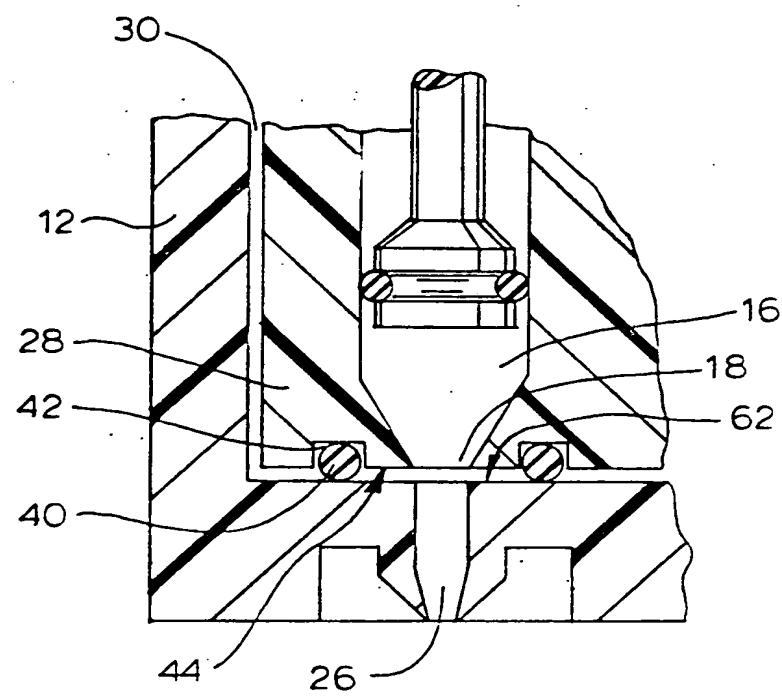


FIG. 9

III. DOCUMENTS CONSIDERED TO BE RELEVANT*

Category*	Citation of Document ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP, A2, 0 482 713 (EASTMAN KODAK) 29 April 1992 (29.04.92), abstract; fig.. --	1, 6, 7, 12
A	US, A, 5 019 348 (OHMS) 28 May 1991 (28.05.91), abstract; fig.. --	1, 6, 7, 12
A	US, A, 2 961 228 (MOORE) 22 November 1960 (22.11.60), claim; fig. (cited in the application). ----	1, 6, 7, 12

- * Special categories of cited documents:¹⁰
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search
03 December 1993

Date of Mailing of this International Search Report

23.12.93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

NARDAI e.h.

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/US 93/07951 SAE 78585

In diesem Anhang sind die Mitglieder
der Patentfamilien der im obenge-
nannten internationalen Recherchenbericht
angeführten Patentdokumente angegeben.
Diese Angaben dienen nur zur Unter-
stützung und erfolgen ohne Gewähr.

This Annex lists the patent family
members relating to the patent documents
cited in the above-mentioned inter-
national search report. The Office is
in no way liable for these particulars
which are given merely for the purpose
of information.

La présente annexe indique les
membres de la famille de brevets
relatifs aux documents de brevets cités
dans le rapport de recherche inter-
national visée ci-dessus. Les renseigne-
ments fournis sont donnés à titre indica-
tif et n'engagent pas la responsabilité
de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 482713	29-04-92	CA AA 2046523 EP A2 482713 JP A2 4365471 US A 5133938	26-04-92 25-11-92 17-12-92 28-07-92
US A 5019348	28-08-91	keine - none - rien	
US A 2961228		keine - none - rien	

